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INVESTIGATIONS CONCERNING THE ETIOLOGY OF SMALL-POX.[1]

BY J. CHRISTIAN BAY.

[With plate XXIX.]

The etiology of small-pox is one of the most interesting problems in bacteriology, and has been subject of considerable investigation for thirty years and more. A brief historical sketch, illustrating what has hitherto been done in this line should, naturally, precede this preliminary record of my own work the progress of which may be traced in the Iowa Health Bulletin published by the State Board of Health of Iowa under whose authority these investigations were carried out during the past year.

Numerous writers have investigated the small-pox and vaccine lymph, and some have recognized specific micro-organisms, both animal and vegetable, as the primary cause of the disease, or of the specific eruptions.

One of the micro-organisms, heretofore more or less generally recognized as the effective agent is the *Micrococcus vaccinæ* and *variolæ;* Bareggi who, among others, studied these, states[2] that the micro-organisms of small-pox and those of vaccine are identical.[3]

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[1] Published in abstracted form in the Medical News, January 26, 1895. Presented to the Iowa State Board of Health, February, 1895, and read before the Des Moines Academy of Sciences.

[2] Sul microbi specifici del vajuolo, del vaccino e della varicella. Gaz. med. Ital. Lomb. Milano (8) VI, 480, 506, 519, 529, 545; with plate.

In 1868, Chauveau[4] proved that vaccine virus is deprived of its active substance by filtration. Hence, it became more than probable that the contagion was a living organism, and no gaseous or diffusible product. " For when he carefully poured a stratum of water upon a layer of lymph, in tiny tubes, he obtained a diffusion of the dissolved material into the water, but this clear solution could not produce pustules like the insoluble residue."

In the same year, Hallier[5] described micrococci " of a singular appearance from human small-pox, cow-pox and vaccine eruptions, the diameter of these bacteria being $\frac{1}{300}'''$ to $\frac{1}{150}'''$; they exhibited motion except when covering the lymph-particles.

Previous to this, G. Simon[6] found, in human small-pox, round particles which were insoluble in acetic acid. Salisbury[7] also claimed to have demonstrated a specific small-pox organism which he named *Jos variolosa ;* it was described as quite polymorphous; its alga-stage was seen in cow-pox eruptions; " fructification " was reached in small-pox eruptions.

Luginbuehl[8] discovered, in sections cleared with acetic acid micrococci which formed colonies at certain places in the skin, near the epidermis, in cases of small-pox eruptions. Beale[9] found " vast multitude of minute particles of living matter or bioplasm " in the small-pox vesicles, but he did not attribute to these the name of *causa morbi.*

Cohn[10] showed the presence of minute cocci in vaccinia and small-pox lymph; when the lymph is fresh, the cocci were moving freely, propagated themselves by division, and, after

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[3] Confer Crookshank, Manual, p. 203; Klein, Micro-Organisms and disease, pp. 79-80.

[4] Comptes Rendus LXVI, 289, 317, 1868.

[5] Aerztl. Intelligenzbl. XV, 75; Virchow's Archiv XLII, 309, 1868.

[6] Müller's Archiv, 1846, 185.

[7] Schmidt's Jahrbücher, 1871.

[8] Verhandl. d. phys. med. Ges. in Würzb. IV, 99, 114; 1873, w. pl.

[9] Disease-germs, their nat. and orig., 1872, 148; pl. XVIII, fig. 64.

[10] Virchow's Archiv LV, 229-238, 1872.

16–32 hours of cultivation, aggregated in masses, afterwards in films the formation of which seemed to be the terminal phase of their life-history.[11] Cohn named this organism *Microsphæria vaccinæ* which was a specific coccus and no representative of some stage of development of some other organism. The name was later changed into *Micrococcus vaccinæ* which Cohn, in his system of bacteriology, described in the following way[12]: "Cells ball-shaped, 0.5–0.75 μ. in diameter, or united two and two or more in chains and masses, also forming a zoogloea. In fresh lymph from cow-pox and small-pox as well as in the pustules in confluent variola."

Weigert, a short time before Cohn, found[13] "vessel-shaped, irregular, often ramified formations of 0.1–0.2 mm. in diameter with granulated, well-marked contents which was not affected by acetic acid, sodium and glycerin. He interpreted these formations as lymphatics filled with bacteria. They were found in the neighborhood of small-pox pustules, and at their edges, where also haemorrhagical herds, and arteries with the same contents were observed. Cohn declared that Weigert's granules were identical with his *Microsphæria*.

Thus it was beyond doubt that vaccinia, cow-pox and variola were caused by attacks of bacteria. Burdon-Sanderson also confirmed this view. The history of the cases also show that the disease is caused not only by a *contagium fixum*, but also by a *contagium halituosum*.

Weigert's observations concerning the lymphatics were repeated and confirmed by Klein.[14]

Klebs[15] set forth the statement that the organism (microcci) in vaccinia and variola exhibit peculiar physiological and morphological properties. The cells are placed four and four together and assume, ontogenetically, no other shape than that of the coccus. The size of the cell diameter was 0.5 μ. This organism received the name *Micrococcus quadrigeminus.* The literature on hand does not elucidate whether this bacterium had, by virtue of its characteristics, any diagnostic value.[16]

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[11] The same aggregations had been observed by Keber.

[12] Beitr. zur Biol. d. Pflanzen, Vol. I, part II, 161.

[13] Ueber Bakterien in der Pockenhaut. Centralbl. f. d. med. Wiss. IX, 606–611, 1871. Ueber pockenaehnl. Eruptionen in innern Organen. Deutsche Zeitschrift f. prakt. Med. I, 367–369, 1874. Anatom. Beitr. z. Lehre von den Pocken, part I, 1874.

[14] Phil. Trans. Lond., 1874; Micro-Organism and disease, 1886, 69.

[15] Arch. f. experiment. Pathol. und Pharm. X, 222, 1879.

In 1883, C. Quist found that vaccine lymph could be artifically propagated in various nutritive media,[17] but such a dilution of the lymph had nothing to do with the bacteria, so far as these experiments went. It is undisputable that Quist propagated the vaccine *virus* along with the dilution of the lymph; the preservation of the virus in glycerin and other media, as done by practitioners, is, therefore, in spite of Pfeiffer's views, no simplification of Quist's method, in as much as propagation and preservation of efficacy (life activity) are not absolutely identical. Small-pox is unquestionably a bacterial disease, and we know that bacteria can live without propagating themselves; the ultimum temperature of propogation is lower than that of life, in both directions from zero.

Pfeiffer[18] found, in 1885, a sprouting fungus which he named *Saccharomyces* seu *Cryptokokkus vaccinæ vaccarum.* This fungus is not very much different from the so-called *Saccharomyces apiculatus,* and is no *Saccharomyces*[19], as it belongs to the group *Torula* in the sense of Pasteur and Hansen. In small-pox lymph, I have occasionally met a *Torula* which corresponds to Hansen's fifth species.[20] Pfeiffer's fungus did not bear endospores, and has no causal relation to small-pox. This *Torula* as well as the saprophytic bacteria, and the animalculæ which Pfeiffer reported from pustules will appear in many other eruptions and ulcerations. It appears that some of Pfeiffer's drawings[21] as well as Beale's " bioplasts (loc. cit.) indicate serious misinterpretations of the microscopic pictures.

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[16] Conf. Lœffler, Vorles. ueb. d. gesch. Entwickelung der Lehre von den Bakterien I, 132, 1887.

[17] Finska läk. sällsk. handlingar XXV, 271, 1883. XXV, 341, 1883. Berl. klin. Wochenschr., 1883, 811–813. Hygiea (Stockholm) XLVI, 194, 203, 1884. See also Medical News.

[18] Correspondenzblatt d. allgem. aertzl. Vereins von Thüringen., 1885. No. 3. Sep. 12 pp.

[19] See my paper in THE AMERICAN NATURALIST, XXVII, 685–696, 1893.

[20] See Jœrgensen, Micro-Organisms and Fermentation, 1893, p. 190, and Bay, Amer. Monthly Microscop. Journal, XV, 42; 1894.

L. Voigt described, in 1885,[22] three different forms of cocci from small-pox pustules. All of them would liquefy gelatine, and one of them was considered the probable carrier of the contagion. No definite results were, however, obtained. There were two cocci, and a diplococcus.

Pohl-Pincus also studied the micrococci found in specific eruptions, and showed their passage through the epidermis of a calf after inoculation.[23]

Hlava[24], Bowen and Garrê have succeeded in isolating a streptococcus (*Streptococcus pyogenes*). They considered the united attack by these pyogenic cocci the cause of the disease. Koch and Feiler were, however, of the opinion that although some of the saprophytic micro-organisms found in vaccine lymph are pathogenic, they do not carry the contagion.

Protopopoff[25] succeeded in finding a streptococcus which corresponds, both macro- and microscopically, to the descriptions of the *Streptoccocus pyogenes.* Samples from pure cultures were injected in rabbits, dogs and cats, but without effect. Although this does not imply that this organism cannot affect man, it seems improbable that it could have any causal relation to variola.

Crookshank[26] and Copeman[27] found, in vaccine lymph, great numbers of common saprophytic and of some pathogenic bacteria, but no specific organism.

Rille[28] observed cocci in the vesicles and blood of persons suffering from varicella, but did not apply himself to bacteriological studies of these organisms.

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[21] Correspondenzblatt d. allg. aerztl. Vereins von Thüringen, 1887, No. 2, Sep. 12 pp. 2 plates. Monatshefte f. prakt. Dermatologie, VI, 1887, No. 10. Sep. 13 pp. 2 pl. Die Protozoen als Krankheitserreger. Jena, 1890.

[22] Deutsche med. Wochenschrift, XI, 895–897, 1885.

[23] Pohl-Pincus, Untersuch. neb. d. Wirkungsweise der Vaccination, 1882.

[24] Sbornik Lékarsky, II, 96–105, 1887. Cblt. f. Bakt. II, 688, 1887.

[25] Zeitschrift für Heilkunde XI, part 2, 1890. Sep. 7 pp.

[26] Transact. Seventh Internat. Congr. of Hyg. and Dermogr. II, 326, 1892.

[27] *Ibidem*, 319–326.

[28] Wiener klinische Wochenschrift, No. 38–39, 1889.

Probably Sternberg was right in stating[29] that the etiology of small-pox is still undetermined. Still, some of the investigations above cited furnish very interesting points which are of value to those who wish to reinvestigate the matter.

Micrococci of different shape and characters are, however, not the only bacteria which have been observed in small-pox and vaccinia. A few statements point towards the presence of other bacteria, namely, bacilli. Crookshank (loc. cit.) mentions that he has found *Bacillus pyocyaneus*, *B. subtilis*, different *Bacterium*-forms (one yellow), and a bacillus resembling *Bacillus subtilis*. Martin[30] has described a bacillus of vaccine lymph. The ends of this bacillus are round or square, and it may form micrococci (!) which are arranged in chains of five or six cells. The author admits the possibility that both a bacillus and a micrococcus were present.

Coze, Feltz and Baudoin[31] have demonstrated the presence of bacilli in the blood of variola; upon injections of this blood into the veins of a rabbit, the typical symptoms of variola were produced.

In sheep-pox lymph examined by Zimmermann[32] three bacilli were found one of which had almost the same appearance as *Bacillus amylobacter*. A second investigation showed the presence of a short-limbed bacillus; *Micrococcus vaccinæ* (or *variolæ*) occurred in both series of investigations. All of Plaut's plates demonstrate bacilli which he was able to cultivate.

Toussaint's studies which also resulted in a discovery of bacilli are mentioned by Plaut (loc. cit.)

In April, 1894, vaccine "points" were procured from Dr. Hewitt's Vaccine Station at Red Wing, Minn. A watery dilution of the lymph adhering to the "point" contained, when examined by 1160 diam. m. (Bausch and Lomb, Oc. C2, Obj. $\frac{1}{12}$ oil imm.) a few amorphous bodies which assume a yellow color with IIKa, a few round bodies and irregular masses (probably nuclei or fragments of cells), dispersed in a clear fluid. I could distinguish no micrococci or other bacteria, and no staining revealed any living organisms. Some of the round bodies observed in ten different examinations may have been spores or micrococci, but their nature was not revealed by the microscope.

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[29] Manual of Bacteriology, 1892, 528–529.

[30] Boston Med. and Surg. Journal, CXXIX, 589, 1893.

[31] *Fide* Magnin-Sternberg, Bacteria, 1884; 410, 464.

[32] Plaut, Das organisirte Contagium der Schafpocken, 1882; 22.

A series of plate cultures upon " Pasteur gelatine "[33] was then arranged, but there occurred no development. These plates were prepared from 10 parts of gelatine to 90 parts of Pasteur's fluid. So, test-tube cultures in Pasteur's fluid alone, and in bouillon (beef; one pound of meat to one liter of water) rendered alkaline by Cl Na. were made. The points were grasped with a forceps, passed through a flame, and dropped into the medium which had been, previously, submitted to a very thorough fractional sterilization, as by the usual preparation of medium supplies. Great care was exerted in order that no infection from without should take place.

By a temperature of 24°C. the culture fluid would, on the next day after inoculation, become slightly turbid; on the second day the turbidity increased, a thin film being formed on the surface, and on the third day a grayish, highly tenacious film made its appearance. Microscopic investigation showed the presence of *bacilli*. The latter are colorless; they exhibit no motion, are devoid of cilia; their long diameter measures 0.6–1.0 μ and the short diameter .2–.3 μ. During the first and second days, they seem to develop in colonies of 20–200 cells, although, under the cover, many cells appear to be free and isolated.

The zooglœa (surface-film) has, to a great extent, the same appearance as the film-growth of the yeast-like *Mycoderma*, being folded, and of a greasy appearance. It is so tenacious that it resists the weight of the column of the culture medium which was observed as one of the cultures chanced to be inverted. Its connection with the culture vessel is quite intimate. On the fourth days, fragments of the zooglœa began to descend to the bottom, and the macroscopic appearance of the culture remained, after this, unaltered for three weeks and more. During this period, however, the microscopic appearance of the bacillus was gradually much modified.

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[33] See Salomonsen, Bacteriological Technology, pp. 460 and 464.

This organism was found, with three exceptions, in 65 cultures from vaccine points hitherto made. Buttersack whose recent investigations will be mentioned in due time ventures the supposition that the specific organism of vaccine was not hitherto detected, because of its index of refraction being identical with that of the medium (lymph). I see no reason for this supposition, and I am prepared to explain Buttersack's theory from my own observations.

This bacillus has, to a great extent, the same appearance as those found by Plaut[34] and Zimmermann in sheep-pox.

Already at the beginning of the development, while the medium is well stored with nutrition, *the bacilli* bear spores. This being the most conspicuous feature of the organism, I named it *Dispora variolæ.* The systematic side of the description is as follows:

Genus: DISPORA.

Dispora: Kern, 1882.

Kern (Botanische Zeitung, 1882, No. 16) founded this genus upon one species which was found in kephir and which was characteristic mainly by having two spores in each cell. The genus belonged to the bacillus-group. Kern's *D. caucasica* has not been rediscovered by later students of the kephir-organisms (Beyerinck, M. Ward, Mix), and the genus-name vanished into *Bacillus* (Crookshank, Manual, 312).

Dispora variolæ.

Syn. The spore stage was described under the following names: *Microsphæria vaccinæ* Cohn, *Micrococcus vaccinæ* and *variolæ* Cohn, *Jos variolosa* Salisbury.

Habitat: In vaccine and small-pox lymph constant. Descr. Bacilli 0.6–1.0 μ by 0.2–0.3 μ. Two spores in each cell, one at each end. Aërobic.

On the sixth days of cultivation, *free* spores begin to make their appearance, both in the fluid and in the zooglœa. They are globular, highly refractive, and may be mistaken for what appeared to me, by a little over 2000 d. m., as vacuoles. The latter are, however, larger, and their shape is oval or rectangular.

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[34] Loc. cit. Beilage I–IV b; especially II a.

The same organism was found also in the lymph of variola confluens kindly furnished by the small-pox hospital in Chicago. Out of forty bouillon-cultures made from this lymph, only two failed to show the presence of the *Dispora.*

To prove that *Dispora variolæ* was not accidentally caught in the cultures from the atmosphere, gelatine-plates (10% gel., 90% beef-bouillon) were exposed to the air at the tables and windows for different periods of time. Among the numerous organisms thus obtained, none presented the characteristics of the above named bacillus

When cultures were examined on the eighth day after inoculation, the cells seemed to be crowded together in separate masses, each cell being surrounded by a rather thick layer of a gelatinous mass, free spores being abundant. As the cultures grew older, the cells gradually became more and more lengthened, forming rows, and on the fourteenth and fifteenth days, the culture presented the appearance shown in fig. 4. The cells were lengthened and formed long, thin threads. Spores were abundant, both in the cells and free. The number of cells was now gradually diminished, and, on the thirtieth day, very few were seen, the number of spores being altogether predominating. When traces of this last stage of development were transferred, with the usual precautions, into new medium, development promptly followed, as above described.

The following method of staining gave good results: A small drop of the culture was placed between two covers and slightly pressed between them. The covers being separated in the usual way were placed, moist side upwards, under a bell glass. When some of the fluid had evaporated, the clean side of the covers were placed three times, for a period of about one-second, in the immediate neighborhood of a flame. When completely dried in the temperature of the room, the covers were placed in alcohol for two or three minutes, and again dried; then they were floated, film-side down, upon aniline blue or aniline violet for 24 hours, washed, dried and mounted in the usual way.

While this organism had the appearance of being a specific bacillus-form, I was not thoroughly convinced thereof until I had made a fractional culture in bouillon which resulted in the development of the one form described. The *Micrococcus vaccinæ* I have never found in vaccine or small-pox lymph.

Regarding the polymorphism of this species I can state that I have observed no such swellings at the middle or ends of the long cells in old cultures as Martin (l. c.) noticed in the bacilli found by him, or as Hansen[35] described for acetic bacteria.

From the figures of *Micrococcus vaccinæ* and *variolæ* which I have seen I am inclined to believe that this organism is not specific, but consists of free spores of *Dispora variolæ.* I also believe that the facts in regard to the spread of small-pox, as well as the observations stated above point towards the conclusion that the spores are the main source through which the disease, itself, as well as vaccinia, are reproduced.

The organisms from small-pox and vaccine lymph are morphologically identical. The physiological difference consists mainly in the attenuation of the form found in vaccine lymph, so far as has been hitherto ascertained.

Buttersack[36] published, a short time ago, an account of certain bodies which occurred, constantly, in vaccine lymph, and which may have some relation to vaccinia. He allowed lymph to dry on covers; having fixed the latter to the slides by means of bees-wax, he inspected the film by immersion and observed a net-work of threads wiih small, refractive, round bodies. Landmann[37] and Dräer[38] interpreted Buttersack's discovery as threads of fibrin and other albuminates. I would assume that B. had seen the "thread-stage" of the organism found by me. Having not yet seen B's illustrations, this is a mere supposition.

The diagnostic value of my discovery is yet uncertain. I hope to be able to report upon the progress of the work, especially concerning inoculations upon animals and the preparation of vaccine in the laboratory, at some future time, when the work now in progress, has reached completion.

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[35] Comp. Rend. Laboratoire de Carlsberg III, 265–327, 1894.

[36] Arbeiten a. d. Kais. Gesundheitsamte IX, 96–110, 1894.

[37] Hygienische Rundschau, 1894, 433–34.

[38] Centralblatt f. Bakt. und Parasitenkunde XVI, 561–564, 1894.

Bacteriological Laboratory, State Board of Health. Des Moines, Iowa, February, 1895.

EXPLANATION OF PLATE XXIX.

Fig. 1. $\frac{1000}{1}$. *Dispora variolæ,* two days old growth in Pasteur's fluid.

Fig. 2. $\frac{1000}{1}$. Same; four days old. Specimen from surface film.

Fig. 3. ca. $\frac{1500}{1}$. Same; eight days old culture in bouillon. A few spore-bearing cells.

Fig. 4. ca. $\frac{1500}{1}$. Same; eleven days old culture in bouillon. Spore-bearing cells numerous.

Fig. 5. $\frac{1000}{1}$. Same; 25 days old bouillon-culture. Some free spores; chains.

Fig. 6. $\frac{600}{1}$. Same; one month old bouillon-culture. Cells almost disappeared; free spores in excessive numbers.

THE AFFINITIES OF THE LEPIDOPTEROUS WING.

BY VERNON L. KELLOGG.

It has long been recognized that the venation of the wings of the Trichoptera and Lepidoptera is of similar general character; and recognized, too, although less popularly, that the genera *Hepialus* and *Micropteryx* display more clearly than do any other lepidopterous forms this general resemblance to the trichopterous venation. Speyer,[1] in 1870, pointed this out in his discussion of the affinities of the Lepidoptera and the Phryganidæ. His too serious consideration of the many mere analogies apparent in any comparison of the groups did much

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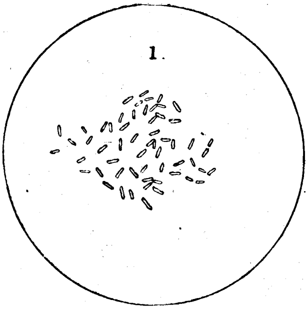
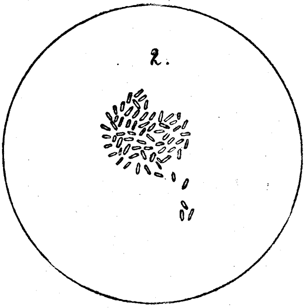
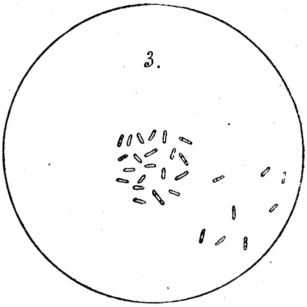
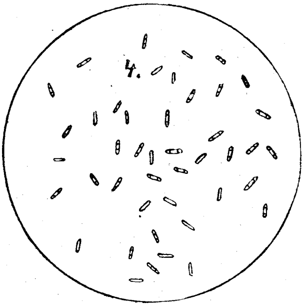
[1] Speyer, A. Ueber die Genealogie der Schmetterlinge, Stettiner Entomologische Zeitung, pp. 202–223, 1870.

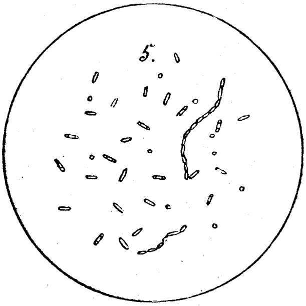
PLATE XXIX.

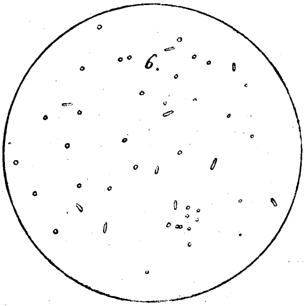












Bay on Small-Pox.